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The long-term goals of this project have been to develop new strategies for the use of oligonucleotides tagged with multiple fluorescent labels as biosensing probes of specific nucleic acid sequences. First, we have designed new classes of fluorescent labels for DNA. Second, we have developed novel modes of interaction between fluorescent labels, leading to color changes on detection of nucleic acids Finally, we have developed new chemistries for joining DNA strands which can be applied to color-changing strategies. These lines of research led to useful developments in biosensing, and have opened up new areas of investigation for the future.

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FINAL PROGRESS REPORT

GRANT # DAAD19-00-1-0363-P00001

TITLE: **Interactions of Multiple Fluorescent Labels in Biological Sensing**

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BODY OF FINAL REPORT

The information in this document is proprietary to the University of Rochester and to Stanford University.

GRANT # DAAD19-00-1-0363-P00001

TITLE: **Interactions of Multiple Fluorescent Labels in Biological Sensing**

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INTRODUCTION

This report covers the total award period for this grant (years 2000-2003). This project has been ongoing at Stanford University for three years. The chief aims of the project were:

Aim 1. **Testing multifluorophore interactions in new nucleoside-dye chimeras.**
Aim 2. **Covalent capture of excimers in RNA sensing**
Aim 3. **Energy transfer through multiple dyes.**
Aim 4. **Optimizing energy transfer from excimers to FRET acceptors.**
Aim 5. **Development of fluorescence color-change sensors based on excimers and energy transfer.**

FINAL TECHNICAL PROGRESS REPORT

In the third year of this project we have made significant progress on several proposed tasks, with strongest progress in Aims 1,2,3 and 5. Below are described details of our work.

AIM 1: Testing multifluorophore interactions in new nucleoside-dye chimeras.

Our project involved discovering and developing the interactions between two or more fluorophores in color-changing probes of RNAs and DNAs. Such probes could eventually be useful in sensing of RNAs and DNAs associated with pathogenic bacteria and viruses, and distinguishing between single-nucleotide variations such as occur in mutants of viruses or the natural variations that occur between humans.

Our Aim #1, has been to construct new fluorophores in which the flat fluorescent species replaces the DNA base. Over the whole grant period we developed five such ne wmolecules. This includes fluorescent nucleosides containing dimethylaminostilbene, dimethylaminobenzene, benzopyrene, hydroxyperylene, and porphyrin replacing the DNA base.

AIM 2: Covalent capture of excimers in RNA sensing.

In this Aim we attempted to find ways to make color changes (involving two dyes) permanent after sensing DNA and RNA sequences. We described an "autoligation reaction" that can join DNA strands after a target sequence is sensed. During the grant period we described progress towards the use of pyrene and/or perylene in self-ligating DNA probes. The plan was to elicit a color change in the product, thus reporting on the presence of a specific genetic sequence. We did, in fact, make the desired pyrene-based molecules. Unfortunately, however, they did not ligate, presumably because they bind in a geometry that does not support the reaction.

However, we did develop an entirely new strategy for labeling nucleic acids with excimers. We found an enzyme, TdT, that can incorporate several excimer-forming dyes into one strand of RNA or DNA. We optimized this reaction and characterized the products. The enzyme can incorporate four successive pyrene nucleosides onto the end of a DNA strand, resulting in a green-white excimer label. We successfully observed the labeled products on polymer beads, and we are now assessing whether such a reaction can be used in human cells to detect apoptosis (programmed cell death).

AIM 3: Energy transfer through multiple dyes.

Our long-term goal in this Aim was to evaluate the possibility that three or more dyes can undergo energy transfer phenomena. In this approach, the dye having the highest energy absorption is excited, then rather than re-emitting a photon it transfers its energy to a second dye, which in turn transfers to a third. This last dye then emits light at a redder (lower energy) wavelength. This multi-transfer approach offers unusual characteristics such as exceptionally long Stokes shifts. During the grant period we did succeed in observing more than one case of multi-dye energy transfer. A manuscript is in the planning stages.

AIM 4: Optimizing energy transfer from excimers to FRET acceptors.

We tested Aim 4 during the grant period by screening combinatorial nucleoside dye libraries with a potential FRET acceptor (HEX) at the end. We then screened the beads that gave the strongest HEX

signals, and identified the polyfluors that were responsible. We then re-synthesized several of these on larger scale for study. Although we did clearly see emission from the HEX dye, we did not observed any enhanced emission, where the polyfluor was gathering the light and transferring it to HEX. One possible reason for this might have been that the HEX was too close to the polyfluors, thus encouraging charge transfer-based quenching. Future studies may address this possibility.

AIM 5: Development of fluorescence color-change sensors based on excimers and energy transfer.

During the grant period we further developed our color-changing probes for detection of DNAs and RNAs. We developed a new strategy for probes that use quenchers, thus "lighting up" in the presence of a genetic target. We demonstrated these by identifying ribosomal RNA sequences in bacteria. This success has encouraged us to follow this goal in future studies.

KEY RESEARCH ACCOMPLISHMENTS, 2000-2003

• We designed and synthesized several new nucleoside fluorophores and quenchers, in which fluorescent (or quencher) molecules replace the DNA base. These have new fluorescence properties that may be useful in conjunction with other dyes, including new absorption and emission wavelengths, and varying ability to undergo exciplex interactions.

• We observed a new form of "excimer" labeling of DNA, using the enzyme TdT to put the unnatural label in place. This type of labeling my be useful in detection of programmed cell death.

• We developed a method using simple color-change phenomena that is coupled to a simple chemical reaction to build highly successful color-changing sensors for RNA and DNA.

• We succeeded in developing new, faster selenium-based chemistry for the joining of DNA strands. This should allow sensing to be carried out more rapidly in the future.

• We described a new and improved RNA sequence-detection method based on quenching and ligation, and have demonstrated it in bacteria..

REPORTABLE OUTCOMES

Seven papers appeared in print during the grant period. Copies were sent previously.

1. Yanzheng Xu and Eric T. Kool, Rapid, selective selenium-mediated autoligation of DNA strands, *J. Am. Chem. Soc*. **2000**, *122*, 9040-9041.
2. Yanzheng Xu, Nilesh B. Karalkar and Eric T. Kool, Nonenzymatic, reagent-free multicolor discrimination of single-base point mutations, *NatureBiotechnology* **2001**, *19*, 148-152.
3. S. Sando and E. T. Kool*, Quencher as leaving group: efficient detection of DNA-joining reactions. *J. Am. Chem. Soc*. **2002**, 124, 2096-2097.
4. Eric T. Kool, Replacing the nucleobases in DNA with designer molecules, *Accounts Chem. Res*. **2002**, *35*, 936.
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6. Jianmin Gao, Christoph Strässler, Deborah C. Tahmassebi, Eric T. Kool*, Libraries of Composite Polyfluors Built from Fluorescent Deoxyribosides, *J. Am. Chem. Soc*. **2002**, *124*, 11590-11591.
7. Hugo Morales and Eric T. Kool*, A Porphyrin C-Nucleoside Incorporated into DNA. *Org. Lett*. **2002**, *12*, 4377.